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December 11, 2001
5402P171US HO/MF-uh

Translation of Document Kern et al.

XP-000929840

Verh.Dtsch.Ges.Path. 81, 611 (1997)

**Coxsackievirus-enhanced endosomolytic gene transfer in contractile cardiac myocytes**

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**Background of the study:** Primary and secondary myocardial diseases (cardiomyopathies) are of great importance in the morbidity and mortality of the population. At present, in the center of the development of molecular genetic concepts for the modulation of endogenic gene activities of cardiac myocytes there is the characterization of heart muscle specific gene transfer systems for the specific application of foreign genes. A limitating factor of DNA/lipidic gene transfer systems relates to the degradation of the internalized DNA in lysosomes and endosomes respectively of transfected cells. By adding of endosomolytic agents, e.g. inactivated viruses or viral peptides, the transfected DNA coming from the endosomes is capable to reach the cytosol more rapidly. Thereby, the endosomal degradation of the transfected DNA is minimized.

**Objective:** Within the context of the development of heart muscle specific gene transfer systems we studied the influence of Coxsackievirus B3 (CVB3) as a potential endosomolytic agent on the efficiency of the transfection of reporter genes in spontaneous contractile cardiac myocytes, including cell-specific promoter/enhancer sequences.

**Methods:** Replication-incompetent CVB3 was obtained by UV radiation and was used for the CVB3-enhanced DOTAP-mediated lipofection of expression vectors in primary spontaneous contractile cardiac myocytes. For the verification of the gene transfection the reporter gene β-galactosidase under control of the human Cytomegalovirus-promoter was expressed. The expression of the reporter gene was detected by means of X-Gal-assays and was quantified luminometrically. Moreover, heart muscle specific vector systems were constructed by using the 5'-regulatory sequence of the cardiac myosin light chain-2 (MLC2) as well as the corresponding 5'-regulatory sequence of the cardiac alphamyosin heavy chain (αMHC).

**Results:** The use of inactivated CVB3 results in a dose-dependent enhancement of the DOTAP-mediated gene transfer. The maximum transfection efficiency was obtained by the use of 10pfu of inactivated CVB3 per cell. The plasmid transfer of the CVB3-enhanced DOTAP-mediated transfection results in an increase of the number of X-Gal positive cells up to six-fold compared to the transfection of the reporter gene construct using exclusively DOTAP. As a result, up to now a transfection efficiency of up to 10 % was achieved in primary cardiac myocytes without inhibiting their spontaneous contractility.

**Conclusion:** These results give evidence for the fact that DNA transfected by replication-incompetent CVB will reach the cytosol more rapidly while coming from the endosomes, whereby the transfection efficiency of the DOTAP-mediated gene transfer in spontaneous contractile myocytes is increased fundamentally. The Coxsackievirus-enhanced endosomolytic gene transfer does not interfere with the spontaneous contractility of cardiac myocytes by which an important requirement for the modulation of the endogenous gene activity of cardiac myocytes is fulfilled.

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